STUDIES ON THE UTILIZATION OF ELECTRO-SMOKES-SOLUTION FOR FISH SMOKING

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by

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Although smoked fish are generally utilized in western countries as conservative and delicious food, their popularity was far behind in our country, because of their cost as compared with other marine foods and for not being so favourable for rice meals as regards their flavor and taste. The so-called smoked foods are the recently imported materials from Europe, however, it is also considered that they should be included into the category of "Katsuo-bushi" in Japan, which is made by the steaming, smoking and drying of bonito meat.

After World War II, the demand for smoked foods gradually increased with the improvement of dietary habits through the popularity of bread meal in Japan.

On the other hand, the strong preservative effects of the chemical substances such as formaldehyde and a number of phenolic compounds which possess bactericidal and antioxidative action, bring a quite important significance to the utilization of perishable marine foods.

Based on the point of these views, various chemical properties and extensive utilization and application to the food manufacture of the Electro-smokes-solution (E.S.S.) invented by Dr. Y. Toriyama, Faculty of Technology, Tohoku University, (1) were studied in our laboratory.

In the present report, the chemical composition and the germicidal and antioxidative properties of some kinds of E.S.S. and their experimental application for the making of smoked fish are described.

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(I) Chemical composition of E.S.S

Materials

E.S.S. was made in Dr. Toriyama's laboratory with the apparatus shown in Fig. 1. Smoke produced from moist saw dust, which was imperfectly burned on the electric heater in the burning chamber, was passed through

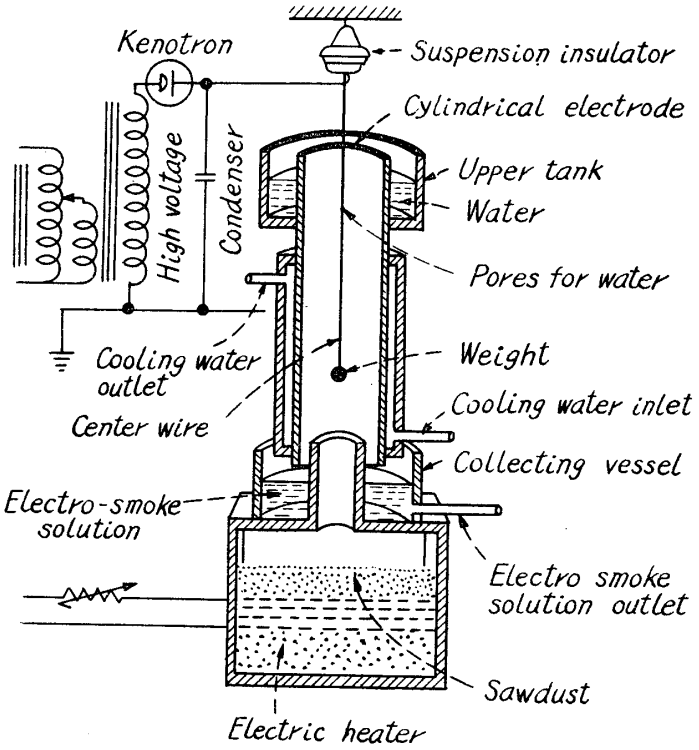

Fig. 1. Apparatus for collecting of Electro-smokes-solution.

the cylindrical collector which consisted of a center wire hung with hanging insulator and cylindrical electrode with the cooling water jacket. D.c. voltage 30–40 Kv. caused smoke and vapor to condense and fall into a well. The smoke consisted of gas and small particles which were charged and ionized negatively, was attracted to the inner positive surface of the cylindrical electrode by the electrostatic action and rinsed into the collecting vessel. The tarry liquid which was composed of the most part of smoky components was then diluted with some water. Then, insoluble substances turbiding in the solution were removed by filtration. So-called E.S.S. was given as liquid which was an 8–10 Baumé transparent, light brown in color and had strong smoke flavor.

Composition

The analytical results of some kinds of E.S.S. are given in Table 1. In

these analysis, formaldehyde was determined after B. Alexander's method (2). Aceton, methanol and resinous acid substance were respectively determined by the method of Messenger (3), W. Ender (4) and Kagaku-kogyo-shiken-ho(5).

Table 1. Chemical composition of some kinds of Electro-smokes-solutoins. (gr. in 100cc. of solution)

Burned materials	Broad leaf	Thorny leaf (A)	Thorny leaf (B)	Orange-rind	Straw
Specific gravity	1.124	1.084	1.046	1.071	1.050
Moisture	73.5	83.5	92.0	85.5	90.0
PH	2.6	2.0	2.4	3.4	3.2
Filtrated residue	1.253	0.462	0.073	0.972	0.666
Ash	2.97	0.52	0.01	0.40	0.78
Total acid (as acetic acid)	5.28	3.39	3.68	3.57	2.17
Formaldehyde	2.21	0.48	0.35	1.46	1.39
Aceton	1.62	0.75	0.83	1.09	0.95
Methanol	1.35	0.36	0.58	1.14	0.98
Resinous acid substance	5.32	2.74	—	3.12	1.15

The compositions of wood smoke were hitherto investigated by M. Saito (6) and Lane (7).

Unfortunately it was impossible to discuss these results directly in comparison with those of the authors because of the differences of the experimental conditions.

It was naturally thought that the different kinds of burned materials should give different chemical compositions on each E.S.S. Although it was impossible to assert clearly what caused it with only these data, there were actually delicate and special flavors according to the kind of burned materials. In addition, the burning conditions were not scrutinized minutely, so that, in this report, the discussions were advanced by pressuming that the burning conditions were almost equal.

At first, these data show that the greater the specific gravity was, the more the ash and filtrated residues and the less the water contained in the solution.

On the other hand, though the contents of methanol and acetone were indicated in the same tendency as ash and filtrated residues, the E.S.S. which contained large amounts of these components was guessed to contain at the same time other elements which should increase the specific-gravity, since such components as methanol and acetone had essentially to decrease the specific gravity. And, in Table 1, ash, acetic acid, formaldehyde and resinous acid substance should correspond with these elements.

On pH value, all of these solutions showed remarkable acidity. Namely, E.S.S. obtained from pine and Cryptomeria (so-called thorny leaf woods)

gave the lowest value of 2.0, and the next was found to be E.S.S. obtained from a broad leaf tree. E.S.S. obtained from straws and orange rinds showed more or less higher values than both mentioned above, those were 3.2 and 3.4 respectively.

In those results, it was very interesting to note that E.S.S. obtained from orange rinds, which were thought to contain greater quantities of acidic substance than the others, showed the highest pH value.

The quantities of formaldehyde, acetone and acidic substances were found to be considerably different between wood smokes and orange rinds or straw smoke.

And the wood smoke, especially the smoke obtained from the broad leaf wood, generally contained greater amounts of these compounds than the others, while the smoke from thorny leaf woods contained rather less amount of them.

The fact that the smoke from the thorny leaf wood had hardly been used for smoking from ancient time should be indorsed by the fact mentioned above. And then, comparing these results with "Mokusaku-eki (destructive distillate)" (8) which had been considered to have properties kindred to E.S.S., "Mokusaku-eki" contained rather more acetic acid and less aldehyde and acetone than any of E.S.S. This fact was very interesting when it was considered that alcohol should be mainly oxidized to aldehyde (9) by means of the effect of corona-discharge during the operating process.

(II) The trial production of smoked fish by application of E.S.S.

In this work the authors tried to manufacture smoked fish by using E.S.S. which had many available properties for smoking of food as mentioned above.

Sauries removed of their head, viscera taken off and washed with water were steeped in various kinds of E.S.S., which were diluted in various concentrations with water, for different periods. After that, those sauries were dried for various periods in a simple gas oven shown in Fig. 2, then the appearance and taste of them were examined.

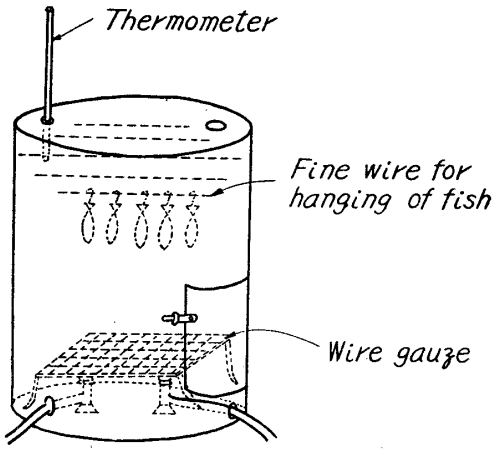

Fig. 2. Simple gas oven.

The results are given in Table 2. In those manufactures thus treated, the best one, which was quite similar to the formal smoked fish, was obtained by the following procedure. Washed cleanly with water, the raw fish were drained for about 50 minutes,

and then steeped for 40–50 minutes in E.S.S. which was diluted with one or two volumes of water, and then dried in an oven for several hours at 60°C or so.

In this procedure, however, it was very important to remove the turbiding substances which occured in the solution during the process of dilution. Those substances might be insoluble tar fractions, so that if they adhered to the surface of fish the appearance of the manufactures would become slightly black in color.

In addition to the above, it should be also noticed that the pretreatment of dehydrating fish by salting and draining so as to have light wrinkle on the fish surface was essential for good appearance of the manufacture. In the comparative examination of the manufactures which were treated with some kinds of E.S.S, though it was necessary to investigate more deeply, the manufacture treated with E.S.S. obtained from the broad leaf tree showed superior quality. Concerning the thorny tree smoke solution it will become suitable for food when the offensive smell and taste are removed.

The utilization of the characteristic smell of E.S.S. obtained from the rind of the orange should be further studied.

Moderate aridity of manufacture, in this process, was a striking point to produce a good quality. Aridity for only two hours at 60°C was sufficient to produce a product kindred to hot smoked fish. To produce more preservative product like cold smoked fish, five or six hours at 60°C was needed.

Although manufacturing of smoked oyster was tried, a favourable result was not obtained.

(III) The preservative properties of smoked sauries treated with E.S.S.

It was indicated, in the above experiment, that E.S.S. contained the most part of the germicidal components in smoke such as formadehyde, alcoholic and phenolic compounds.

So, in the present work, the preservative effect of E.S.S. on fish was investigated.

To evaluate the freshness of smoked fish meat after standing for several days at room temperature, as shown in Table 3, volatile basic substances and formaldehyde contents in it were determined by modified Conway's (10) and by Alexander's method (2)respectively. Parallel with those determinations, the organleptic examinations were carried out. The results are shown in Table 3. It indicates that smoked sauries did not deteriorate, and were suitable for food even after 30 days. The differences of volatile base contents between fresh and treated sauries were about 6–12 mg per cent. The increase of the base did not occur during the standing periods, except the beginning stage

Table 2. Organoleptic examination

Kind of fish	Burned material	Dilution ratio and added material	Steeped period
Sauries	Thorny leaf wood	1) 0 times — 2) 2 〃 — 3) 3 〃 3% NaCl	2 hours 〃 〃
Sauries	Thorny leaf wood	1) 0 〃 — 2) 2 〃 — 3) 3 〃 —	2 hours 〃 〃
Sauries	Thorny leaf wood	1) 3 〃 5% NaCl 2) 3 〃 7% sugar	2.5 hours 〃
Sauries	1) Broad 2) Straw 3) Orange 4) Thorny	2 times 10% NaCl	30 minutes
Sauries	Broad leaf wood	1) 2 times — 2) 3 〃 — 3) 2 〃 5% NaCl 4) 3 〃 〃	i) 1 hour ii) 30 minutes
Sauries (pretreated with NaCl)	Broad leaf wood	3 times	45 minutes
Sauries (frozen)	Thorny leaf wood	1) 0 times — 2) 2 〃 — 3) 3 〃 — 4) 3 〃 1% Ca-acetate and 3% NaCl	2 hours
Herring (salted)	Broad leaf wood	1) 0 times 2) 2 〃 — 3) 2 〃	1 hour
Oyster	Thorny leaf wood	1) 0 times — 2) 2 〃 — 3) 3 〃 — 4) 2 〃 3% NaCl 5) 3 〃 5% NaCl	1 hour
Oyster	Thorny leaf wood	1) 0 times — 2) 2 〃 — 3) 3 〃 — 4) 2 〃 3% NaCl 5) 3 〃 5% NaCl 6) 10 〃 — 7) 20 〃 —	i) a moment ii) 5 minutes iii) 10 〃 iv) 20 〃 v) 30 〃
Oyster	Thorny leaf wood	1) 2 times — 2) 3 〃 — 3) 10 〃 — 4) 10 〃 10% sugar 5) 20 〃 — 6) 3 〃 5% NaCl	i) 1 minute ii) 5 〃 iii) 10 〃

of trial production.

Drying temp. and period	Appearance	Taste and Flavor	Remarks
60～65°C 3.5 hours	D C C	D C C	Generally, the drying was insufficient.
40°C 1 hour, 50°C 1.5 hours, 65～70°C 1 hour and 65～70°C 30 minutes	D D A	D B C	The aridity was adequate.
16 hours at room temp., 55°C 4 hours and 60°C 2 hours	B A	C C	〃
45°C 2 hours and 60°C 3 hours	B B B B	B D C B	〃 Especially, appearance was given by fat sauries.
60～65°C 5 hours and 65°C 1 hour	B B B B	C B B B	〃
60°C 5 hours and 65～70°C 1 hour	A	A	〃
46°C 1 hour and 65°C 5 hours	D D A D	D D D D	1), 2) and 3) were in adequate aridity.
60°C 5 hours	D B A	D B A	It was needed more elaborate aridity for fat suries.
75°C 1.5 hours	D D D D D	D D D D D	The aridity was adequate.
60°C 40 minutes	i) : 1), 2), 4)... 3), 5), 6), 7)... B ii) : 〃 iii) : 〃 iv) v) : D	C C C B except 7) D	〃
60°C 35 minutes	i) : B ii) : 3)... B others... D iii) : D	B C C D	〃 A pretreatment of boiling was carried out before steeping.

The evaluation mark : A : Excellent B : Good
C : Not so good D : Bad

of the treatment, because no difference of volatile base could be found between the smoked sauries after 10 or 20 days and 30 days.

As the factor caused such preservative effect, the aridity of manufacture

Table 3. Preservative property of sauries treated with E.S.S.

No.	Days stored	Vol-N in fresh meat (mg%)	Dilution ratio and added material (times and %)	Vol-N in dry meat (mg%)	Vol-N calculated in raw meat (mg%)	Difference of Vol-N between fresh and raw meat (mg%)	Formaldehyde permeated (γ/100g. of dry meat)
1	10	14.97	0 (thorny)	72.31	24.33	9.36	—
			2	78.10	27.54	12.57	—
			3	76.50	26.11	11.14	—
2	20	16.42	0 (thorny)	61.53	22.80	6.38	110.3
			2	66.82	23.92	7.50	42.6
			3	67.25	24.13	7.71	—
2′	30	〃	0 (thorny)	62.63	23.19	6.77	113.2
			2	62.51	23.14	6.72	93.0
			3	62.63	23.19	6.77	—
3	13	16.71	3-5NaCl	64.55	24.84	8.13	21.4
			3-7 Sugar (Thorny)	68.58	26.35	9.64	35.1
4	13	18.16	2-10NaCl (Broad)	68.55	25.84	7.68	82.2
			2-10NaCl (Straw)	76.39	28.99	10.83	15.8
			2-10NaCl (Orange)	66.61	23.80	5.64	24.5
			2-10NaCl (Thorny)	70.13	26.51	8.35	55.5

In the organoleptic examination, all of them were available as food.

and permeability of germicidal substances in E.S.S. were considered.

Table 3. shows that the quantity of formaldehyde contained in the inner layer of fish tissue treated by steeping in E.S.S. were given as 110, 50–90 and 20–35 γ per cent respectively. Permeability of formaldehyde in E.S.S. into the fish meat was examined with blocks of raw and boiled tuna meat of $10.0 \times 3.5 \times 3.0$ cm^3 in size. That is, these were dried in an oven for five hours at 60°C after steeped in E.S.S. for 60 minutes and the quantities of formaldehyde in each layer of the meat were determined. The results are summarized in Table 4. It clearly indicates that in raw material the amount of formaldehyde contained in fatty meat was less than that of the ordinal one, but in the case of boiled meat it was contrarily larger than in ordinal meat.

Such difference between raw and boiled meat depended upon whether the

fat remained in the tissue. Namely, fat inhibited the permeability of E.S.S. was ousted by boiling and the tissue became so chinky, that the permeation of E.S.S. into the inner layer of tissue was facilitated.

Table 4. Amount of formaldehyde permeated into tuna meat. (γ/gr.)

	Outer layer 0~1.5 mm.	Inner layer 1.5~5.0mm.	Center layer 5.0~10.0 mm.
Raw fatty meat	3.3	0.1	—
Raw ordinal meat	5.6	2.1	—
Boiled fatty meat	19.6	5.6	3.4
Boiled ordinal meat	6.3	2.5	0.1

(IV) Germicidal properties of E.S.S.

Since it was important to determine what component indicated the preservative effect in the above experiment, the germicidal effect of E.S.S. itself and of some components in it were studied.

Germicidal effect of E.S.S. in the sterilized culture medium

Some germicidal components like lower alcoholic, phenolic compounds and aldehyde group in E.S.S. were so volatile that the culture medium was prepared as described below;

Ten cc of the culture medium of hot Bouillon-agar prepared after the ordinary method were poured into test tubes, after corking with cotton they were intermittently sterilized.

Then, 1.0 cc, 0.5 cc, 0.2 cc, ... of E.S.S. were added to each tube to make the concentration of 1/10, 1/20, 1/50,... respectively, and each of them was transfered into Petri dishes to form flat culture grounds. On the surface of the ground, one plutinum loop of organisms suspended in a physiological salt solution was streaked in a zigzag line. After incubation for three days at 31°C., the stages of growth of the bacteria were observed. The results are summerized in Table 5.

The strains used in this work were Proteus-4 and Micrococcus-1 which had been isolated from the shark muscle, and they contained strong urease. The former showed resistance against the germicidal effect of spices and acrids but the latter did not so much.

In this work, the exterior observation on growth stages of microorganisms were exceedingly tried, and it was clearly proved that E.S.S. indicated excellent bactericidal action against Proteus-4 and Micrococcus-1 in the concentration higher than 1/100 and 1/50 respectively.

Table 5. Germicidal property of E.S.S.

Concentration of E.S.S.	Proteus-4			Micrococcus-1		
	Incubation period (day)			Incubation period (day)		
	1	2	3	1	2	3
1/ 10	−	−	−	−	−	−
1/ 20	−	−	−	−	−	−
1/ 50	−	−	−	−	−	−
1/ 75	−	−	−	+	++	++
1/100	−	−	−	++	++	++
1/150	−	+	++	+++	+++	+++
1/200	++	+++	+++	+++	+++	+++
1/500	+++	+++	+++	+++	+++	+++
Blank	+++	+++	+++	+++	+++	+++

The sign of growth stage of microorganism; − : dead, + : slightly developed, ++ : developed, +++ : more developed

Germicidal effect of the sterilized E.S.S. in the culture medium

The aim of the present work was to know clearly whether the germicidal effect of E.S.S. was decreased by means of the volatilization of some germicidal components in E.S.S. during sterilization.

The results given in Table 6 indicate that the sterilized E.S.S. in the

Table 6. Germicidal property of E.S.S.

Concentration of E.S.S.	Proteus-4			Micrococcus-1		
	Incubation period (day)			Incubation period (day)		
	1	2	3	1	2	3
1/ 10	−	−	−	−	−	−
1/ 20	−	−	−	−	−	−
1/ 50	−	++	++	−	+	+
1/100	++	+++	+++	+++	+++	+++
1/150	++	+++	+++	+++	+++	+++
1/200	++	+++	+++	+++	+++	+++
1/500	+++	+++	+++	+++	+++	+++
Blank	+++	+++	+++	+++	+++	+++

culture medium could not inhibit the growth of microorganisms entirely till the concentration become higher than 1/20.

Furthermore, comparing these data with those of Table 5, the germicidal action of E.S.S. decreased to 1/5 against Proteus-4 and to 1/2.5 against Micrococcus through the sterilization. This fact showed that, as it was presumed, the sterilization caused the decrease of the germicidal effect of E.S.S. expelling

some volatile germicidal component from it.

Interpreting the germicidal activity of the volatilized component from the decreased degree of bactericidal effect, these components seem to have kept relatively strong germicidal properties.

Germicidal action of the fractional distillates of E.S.S.

Germicidal effects of two fractions obtained by the fractional distillation of 100 cc of E.S.S. on the heated sand bath were investigated. The properties of those fractions were as follows.

Fraction A;

Below 100°C.: 57cc. transparent liquid, pH 2.4

Fraction B;

100–116°C.: 24.5cc. light yellowish transparent liquid, pH 2.0

These fractions were added into a sterilized Bouillon agar medium and the germicidal effect was examined. The results are presented in Table 7.

Table 7. Germicidal property of fractional distillates.

Species	Concentration	Dist. at lower than 100°C			Dist. at 100~116°C		
		Incubation period (day)			Incubation period (day)		
		1	2	3	1	2	3
Proteus-4	1/ 2	−	−	−	−	−	−
	1/ 3	−	−	−	−	−	−
	1/ 5	−	−	−	−	−	−
	1/ 7	−	+++	+++	−	−	−
	1/ 10	+++	+++	+++	−	−	−
	1/ 20	+++	+++	+++	−	−	−
	1/ 50	+++	+++	+++	++	+++	+++
	1/100	+++	+++	+++	+++	+++	+++
	Blank	+++	+++	+++	+++	+++	+++
Micro-coccus-1	1/ 2	−	−	−	−	−	−
	1/ 3	−	−	−	−	−	−
	1/ 5	−	−	−	−	−	−
	1/ 7	−	−	−	−	−	−
	1/ 10	++	+++	+++	−	−	−
	1/ 20	+++	+++	+++	−	−	−
	1/ 50	+++	+++	+++	−	++	++
	1/100	+++	+++	+++	+++	+++	+++
	Blank	+++	+++	+++	+++	+++	+++

The germicidal activity of fraction A against Proteus-4 and Micrococcus-1 was effective at concentrations higher than 1/5 and 1/7. On the other hand, fraction B was effective even at such low concentration as 1/20 against both of these microorganisms.

Although it was very difficult to compare precisely the germicidal activity of the two fractions since the concentration of effective substances in each

distillate could not be determined, it was clearly shown that both of these distillates had contained some kinds of germicidal components. Especially, referring to the results obtained in the previous experiments, it was interesting that considerable bactericidal activity was shown also in the distillate at low temperature.

Then, when 60 cc. of E.S.S. alkalified by the addition of $Ca(OH)_2$ was distilled at temperature higher than 100°C the blisters occured so violently, that the distillate below 100°C was obtained only 27cc. (pH 5.8), and the germicidal activity of it was investigated. As illustrated in Table 8, the

Table 8. Germicidal property of fractional distillates of alkalified E.S.S.

Concentration of E.S.S.	Proteus-4			Micrococcus-1		
	Incubation period (day)			Incubation period (day)		
	1	2	3	1	2	3
1/ 2	+++	+++	+++	+	+	++
1/ 3	+++	+++	+++	++	++	+++
1/ 5	+++	+++	+++	+++	+++	+++
1/ 7	+++	+++	+++	+++	+++	+++
1/ 10	+++	+++	+++	+++	+++	+++
1/ 20	+++	+++	+++	+++	+++	+++
1/ 50	+++	+++	+++	+++	+++	+++
Blank	+++	+++	+++	+++	+++	+++

fraction was not effective against Proteus-4 but slightly effective against Micrococcus-1 at the concentration of 1/2.

From these results, it is evident that both fractions which were obtained by fractional distillation of acidic E.S.S. had kept the effective bactericidal properties, and although it was thought that fraction A contained many kinds of chemical compounds, such as alcohols, aldehyde groups and organic acids which showed low boiling point, the bactericidal effect of this fraction was mainly due to formaldehyde.

On the one side, the fraction B might contain such bactericidal compounds as formic acid, acetic acid, propionic acid, toluene, xylene, phenol, cresol etc., but it was not clear what the maine effective bactericidal component was.

In the distilled fraction of the alkalified E.S.S., alcohols and basic substance such as pyridine were expected, however, the bactericidal activity of the fraction was not so important after all.

The influences of E.S.S. on pH of culture medium

pH of E.S.S. itself was so acidic that pH of the culture medium would be lowered by the addition of it. From this point of view, it was investigated

how pH of the culture medium should be influenced by the the amount of E.S.S. in the above experement.

As clearly shown in Table 9, pH of the culture medium might be influ-

Table 9. Influence of E.S.S. on pH of culture ground.

Concentration of E.S.S. in culture medium	pH value of culture medium	Growth of micro-organism (after 3 days)
1/ 10	4.4	−
1/ 20	5.0	−
1/ 50	6.4	−
1/ 75	6.4	−
1/100	6.5	−
1/150	6.8	+ +
1/200	7.0	+ + +

enced according to the quantities of added E.S.S. Furthermore the growths of Proteus-4 and Micrococcus-1 were considerably inhibited at pH 4.8 and 5.2 respectively, and at lower pH than that the growths were considerably inhibited.

On the other hand, in the above experiments pH of the culture medium with added E.S.S. were lower than 4.8 only the two cases, namely in concentrations of 1/10 and 1/20. pH value at the limiting concentration (1/100) of E.S.S. as the germicidal agent in the culture medium was 6.5 and not thought to be effective against these microorganisms, so that such effective germicidal property of the medium was not caused by the pH value, but the action of germicidal components in E.S.S.

Germicidal action of tar fraction in E.S.S.

E.S.S. should mainly consist of water and water-soluble substances in the smoke, since insoluble substances had been roughly filtrated out. However, it was also considered that small quantities of tar and resinous acid substance should be contained in the filtrated residue of E.S.S. described in Table 1. In this experiment the germicidal activities of the tar fraction were investigated to confirm the results of the previous experiment. A small quantity of tar was dissolved in alcohol, and the solution was added to the culture medium to make the prescribed concentration of tar.

In addition taking into consideration also upon the influence of the solvent (alcohol) blank test using alcohol was tried parallel to this experiment. The results are given in Table 10. Tar showed a strong germicidal activity. It was effective even at the diluted concentration, as 1/200 against Proteus-4 and 1/1,000 against Micrococcus-1. On the other hand, in the blank test, alcohol in a concentration higher than 6 percent, in other words in a higher

concentration of alcohol in which tar dissolved in ratio of 1 : 150, was effectual on the microorganisms.

Since the effective concentration of tar was so dilute such as 1/200–1/1,000,

Table 10. Germicidal property of tar fraction of E.S.S.

Species	Tar				Methanol			
	Conc. (%)	Incub. period (day)			Conc. (%)	Incub. period (day)		
		1	2	3		1	2	3
Proteus-4	1/ 100	−	−	−	9.00	−	−	+ +
	1/ 150	−	−	−	6.00	+	+ + +	+ + +
	1/ 200	−	−	−	4.50	+ +	+ + +	+ + +
	1/ 500	+ +	+ + +	+ + +	1.80	+ + +	+ + +	+ + +
	1/1,000	+ + +	+ + +	+ + +	0.90	+ + +	+ + +	+ + +
	1/2,000	+ + +	+ + +	+ + +	0.45	+ + +	+ + +	+ + +
	1/5,000	+ + +	+ + +	+ + +	0.18	+ + +	+ + +	+ + +
	Blank	+ + +	+ + +	+ + +	Blank	+ + +	+ + +	+ + +
Micrococcus-1	1/ 100	−	−	−	9.00	−	−	+
	1/ 150	−	−	−	6.00	−	+ +	+ + +
	1/ 200	−	−	−	4.50	+ +	+ + +	+ + +
	1/ 500	−	−	−	1.80	+ +	+ + +	+ + +
	1/1,000	−	−	−	0.90	+ +	+ + +	+ + +
	1/2,000	+ + +	+ + +	+ + +	0.45	+ + +	+ + +	+ + +
	1/5,000	+ + +	+ + +	+ + +	0.18	+ + +	+ + +	+ + +
	Blank	+ + +	+ + +	+ + +	Blank	+ + +	+ + +	+ + +

the influence of alcohol in this experiment might be negligible. It was thought that tar contains heavily many kinds of phenolic compounds and other chemical substances, however the analysis of them was not tried.

However on account of the dark brownish color its availability for the manufacture of foods is questionable.

As mentioned above, it contains germicidal compounds so richly that its utilization must be widely investigated in the future.

(V) Antioxidation of E.S.S.

An excellent advantage of smoking was that the action of the antioxidant in smoke protected the food from fat rancidity. From this point of view, the investigation whether E.S.S. shows the antioxidative action is very important for the utilization of them. The authors, therefore, made smoked sauries by using E.S.S. and its tar fraction and studied on the antioxidative action of them.

Oxidation of fat in smoked sauries made by steeping and spraying of E.S.S.

The head was removed from fresh sauries, the dorsal muscle was opened along the longitudinal line of the body, and the viscera was taken off, after

that they were washed cleanly with 1 per cent solution of salt and the water was drained in the air. Then, they were steeped for 90 minutes in the admixture of salt and E.S.S. or other solution of antioxidants in the rate as shown in Table 11, and dried in the sun. In this experiment, E.S.S. obtained from

Table 11. Admixture of NaCl and E.S.S. or antioxidant.

Solution for steeping of fish (litre)	No. of fish	Steeping period (min.)
4 of admixture of 10% NaCl and E.S.S. in the rate of 1:1	40	90
4 of admixture of 10% NaCl and E.S.S. in the rate of 4:1	40	90
4 of admixture of 10% NaCl and E.S.S. in the rate of 9:1	50	90
4 of admixture of 10% NaCl and Sustain the rate of 99:1	50	90
4 of admixture of 10% NaCl and NDGA in the rate of 99:1	40	90

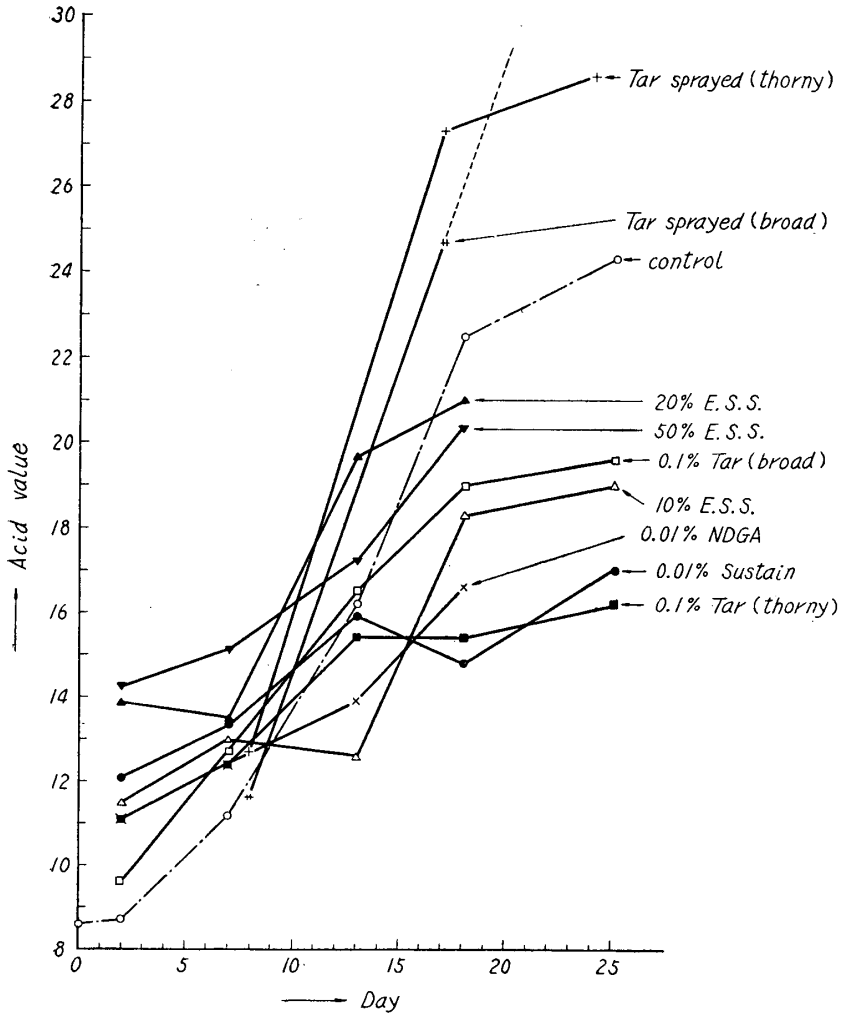

Fig. 3. Antioxidative action of E.S.S. (acid value).

thorny wood saw dust was used.

Beside, the sauries steeped in 10 per cent salt solution for 30 minutes were sprayed with a 10 per cent alcohol solution of tar so that about 0.01 gr. of tar adhered to each one, and then they were dried in the sun. Those wrapped in cellophane bag were stored at room temperature. After various periods of storage as shown in Fig. 3, the contained fat was extracted with ether after dehydrating with anhydrous sodium sulphate. The acid-, iodine- and peroxide value of the fat were determined. The results are shown in Fig. 3, 4 and 5.

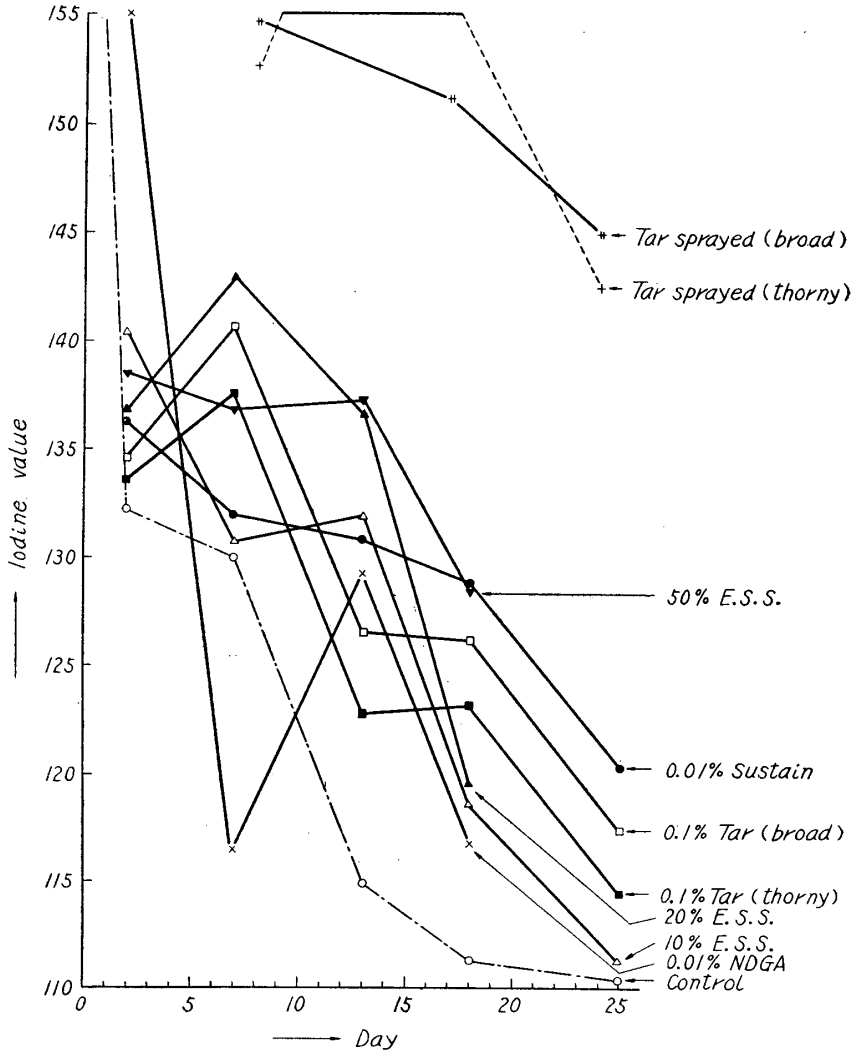

Fig. 4. Antioxidative action of E.S.S. (iodine value).

The acid value of them, except that sprayed with alcohol solution of tar, indicated generally somewhat higher than blank for 10–15 days in the beginning, but generally the value in this period were not so remarkable, and after that the acid value of blank became considerably higher than that of others

tested. It was quite natural to consider that smoked sauries gave higher acid value than others in the beginning period, because the acidic compounds in E.S.S. permeated the body. Considering from the peroxide value and ap-

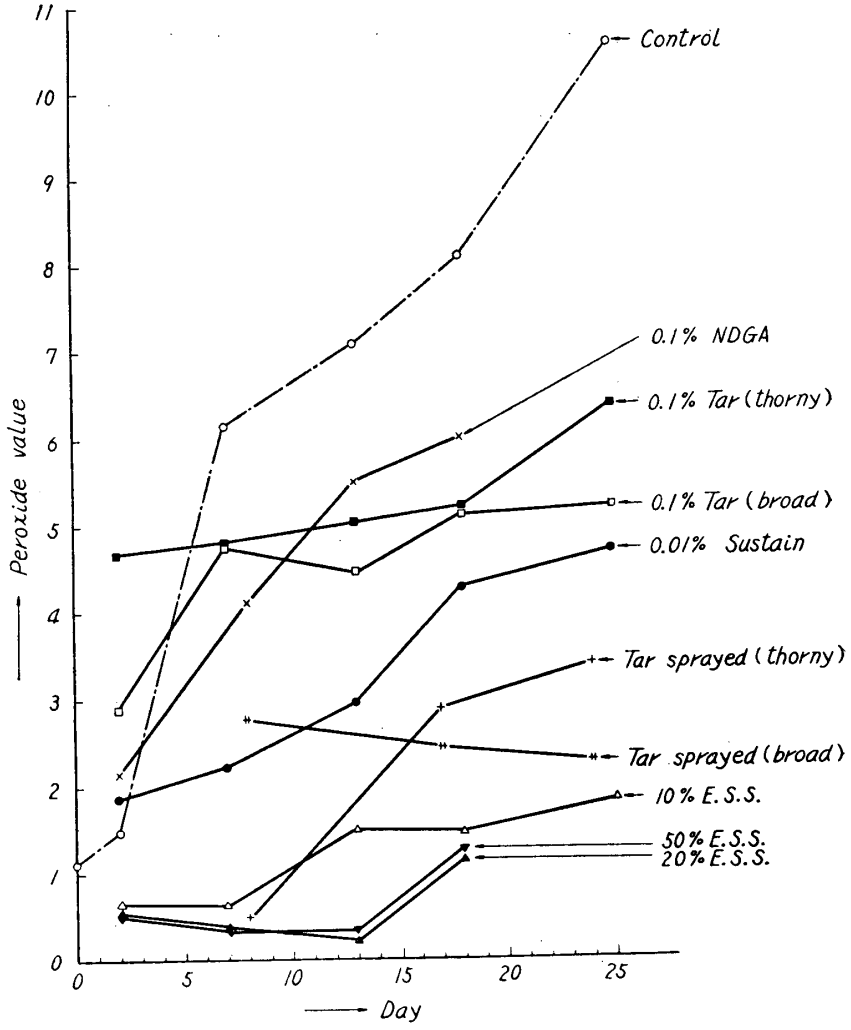

Fig. 5. Antioxidative action of E.S.S. (peroxide value).

pearance of the fish it was definitely shown that E.S.S. and tar were as good as NDGA and Sustain which have been widely used for food as antioxidant. What components in E.S.S. and tars show such antioxidative effect, however, were not pursued.

Antioxidative action of several kinds of E.S.S.

Antioxidative action of several kinds of E.S.S. which were obtained from several different materials such as broad leaf tree, thorny leaf tree, rinds of orange and straws were studied. The method of smoking of fish was as mentioned above.

Fat extracted from smoked sauries preserved for a month or so was used

for the determination of its refractive index, acid value, iodine value and peroxide value.

However in this experiment, the peroxide value was determined after Leay's modified method (11). The results are presented in Table 12.

Table 12. Antioxidative effect of E.S.S. on sauries' oil.

Sample	Refractive index	Acid value	Iodine value	Peroxide value
Broad leaf wood	1.4818	21.2	114.7	14.4
Thorny leaf wood	1.4820	16.0	127.4	33.5
Rind of orange	1.4788	10.1	—	26.7
Straw	1.4800	12.1	113.8	24.9
Blank	1.4824	15.6	110.4	79.6

Smoked sauries indicated lower peroxide value than untreated ones, and their appearance and taste confirmed also that they were protected from the fat rancidity by means of the effect of E.S.S.

Summary

(1) The compositions of several kind of E.S.S. which were made from the broad leaf tree, thorny leaf tree, rind of orange and straw by using Toriyama's collecting equipment of smoke were determined, and some different properties among them were discussed.

(2) Paying attention to the temperature and period for aridity, in this smoking process, it was possible to make a quite kindred product to smoked fish in a short time. Namely, in aridity of fish which were steeped in E.S.S. solution diluted with water two or three times in volume, only two hours at 60°C was sufficient to produce a product kindred to hot smoked fish, and to produce more preservative ones like cold smoked fish only five to six hours at 60°C was needed.

(3) It was illustrated that E.S.S. gave the preservative properties to fish. For instance, smoked fish which were made by this treatment were sufficiently utilized as food even after the storage for 10 to 30 days at room temperature.

(4) The disinfectant substances existed in E.S.S. were pressumed to formaldehyde as a volatile component having low boiling point, and phenolic compounds as nonvolatile components. The latter were richly contained also in the tarry fraction which was removed as the residue in the filtration process.

(5) Fat rancidity of sauries which were steeped in the admixture of salt solution and E.S.S. were studied, and it was confirmed that the oxidative effect of E.S.S. was as remarkable as Sustain and NDGA which have been widely used as antioxidant.

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